

2.3.S.3. CHARACTERISATION

This section contains information specific for presentation Comirnaty Tris-Sucrose Original, which is discontinued. For information purposes, data/information supportive of the platform development approach for other presentations is maintained.

2.3.S.3.1. Elucidation of Structure and Other Characteristics

Section 3.2.S.3.1 Elucidation of Structure and Other Characteristics describes the structure and characteristics of BNT162b2 drug substance (DS) which have been assessed using the analytical approaches outlined in Table 2.3.S.3-1. The analytical methodologies employed for BNT162b2 RNA drug substance characterization are capable of evaluating primary structure, including 5'-capping and 3'-poly(A) tail, and higher order structure. The results demonstrate that BNT162b2 RNA drug substance has the expected structure.

Analytical characterization was performed with BNT162b2 drug substance batch (20Y513C101).

Table 2.3.S.3-1. Characterization Strategy for BNT162B2 Drug Substance

Characteristic	Analytical Approach	Methodology	Section References
Primary structure	Confirm expected RNA sequence at the oligonucleotide level	Reversed phase HPLC-UV and tandem mass spectrometry (LC/MS/MS) – of oligonucleotide fragments generated by RNase T1 digestion	Section 2.3.S.3.1.1.1
	Confirm expected RNA sequence at the oligonucleotide level	CCI Next Generation Sequencing Technology	Section 2.3.S.3.1.1.2
5'-Cap structure	Confirm the 5' capping structure and 5'-end profile	Reversed phase HPLC-UV and mass spectrometry (LC-UV/MS) analysis of purified 5' terminal after RNaseH digestion	Section 2.3.S.3.1.1.3
Poly(A)-tail	Confirm the presence and determine the length of poly(A)-tail	Reversed phase HPLC-UV and mass spectrometry (LC-UV/MS) analysis of purified poly(A)-tail after Ribonuclease T1 digestion	Section 2.3.S.3.1.1.4
Higher order structure (HOS)	Spectroscopic analysis to confirm the presence and fingerprint of HOS	Circular dichroism (CD) spectroscopy	Section 2.3.S.3.1.1.5

2.3.S.3.1.1. Primary Structure

2.3.S.3.1.1.1. LC/MS/MS - Oligonucleotide Mapping

The primary sequence of BNT162b2 DS was analyzed by LC/MS/MS - oligonucleotide mapping. BNT162b2 DS was digested with CCI, and the resulting enzymatic fragments were separated by ion-paired reversed-phase high performance liquid chromatography (IP-RP-HPLC) with UV detection at CCI.

[REDACTED]

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The LC/MS/MS – oligonucleotide mapping results are summarized in Table 3.2.S.3.1-3 and demonstrate that BNT162b2 DS contains the correct sequence as predicted from the linear DNA template (Section 3.2.S.2.3 Control of Materials – Source, History and Generation of Plasmids).

Further details are provided in [Section 3.2.S.3.1 Elucidation of Structure and Other Characteristics](#).

2.3.S.3.1.1.2. Sequencing of RNA

In order to further confirm sequence identity, RNA sequencing for BNT162b2 DS was performed using the CCI Next Generation Sequencing (NGS) technology.

[REDACTED]

[REDACTED]

2.3.S.3.1.1.3. 5'-Cap Characterization by LC-UV/MS

The characterization of the 5' end capped (5'-Cap) and un-capped species of BNT162b2 DS was accomplished by ion-pair reversed-phase high performance liquid chromatography-ultraviolet light detection at CCI and online electrospray ionization mass spectrometry (RP-HPLC/UV-ESI MS) or LC-UV/MS. Sample handling and chromatography follow the method described in Section 3.2.S.4.2 Reversed Phase – High Performance Liquid Chromatography (RP-HPLC).

CCI [REDACTED]

Further details are provided in Section 3.2.S.3.1 Elucidation of Structure and Other Characteristics.

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2.3.S.3.1.1.4. 3' Poly(A)-tail Characterization by LC-UV/MS

Analysis of the 3' polyadenosine tail (poly(A)-tail) of BNT162b2 DS was accomplished by ion-pair reversed-phase high performance liquid chromatography with UV detection at CCI and on-line electrospray ionization mass spectrometry (RP-HPLC-UV/ESI MS or LC-UV/MS). The poly(A)-tail of BNT162b2 DS was cleaved off by ribonuclease T1 (RNase T1) followed by isolation via CCI affinity purification.

Further details are provided in [Section 3.2.S.3.1 Elucidation of Structure and Other Characteristics](#).

The LC-UV/MS results demonstrate that BNT162b2 DS contains the expected poly(A)-tail A30 and L70 segments CCI [REDACTED]

2.3.S.3.1.1.5. Higher Order Structure

The higher order structure of BNT162b2 mRNA DS was characterized in solution using circular dichroism (CD) spectroscopy. A CD spectrum is a measure of differential absorption of the left- and the right-circularly polarized light by the test article, which arises due to structural asymmetry. The ordered structure of mRNA yields a CD spectrum that may contain positive and/or negative signals, while the absence of a CD signal generally indicates a lack of ordered structure.

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